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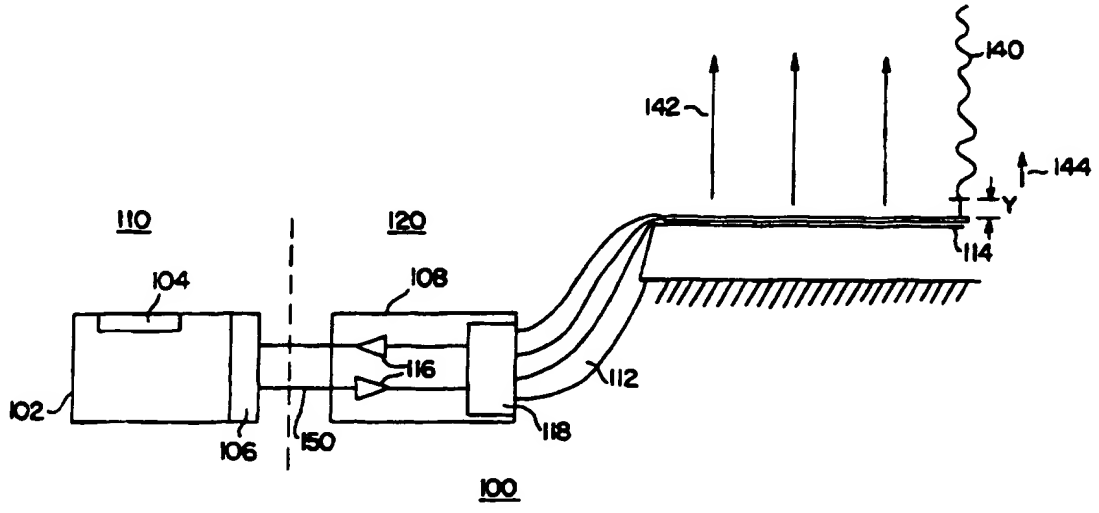

(57) Abstract

The invention relates to methods and apparatus for determining the structure of a biopolymer by piezoelectric force sensing. Biopolymers which can be analyzed include nucleic acids, proteins, polysaccharides and most any sequence of monomers. Biopolymers or biopolymer fragments are attached to a force sensing apparatus and subjected to an electric or magnetic field. The target generates a detectable force on the force sensing apparatus. Force information collected from multiple force sensing elements can be compiled and the structure of the biopolymer determined. Structure information determined may reveal sequence, size, mass or charge information specific for the biopolymer. The invention also relates to methods for the detection of biopolymers in a heterogenous mixture and methods for the detection of a disorder by piezoelectric force sensing. The invention further relates to methods and devices for measuring the mass of a molecular or macromolecular target by analyzing harmonic resonance frequencies.

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PIEZOELECTRIC FORCE SENSING APPARATUS AND METHODS FOR BIOPOLYMER SEQUENCING

Background of the Invention

1. Field of the Invention

The present invention relates to methods and apparatus for the sequencing of biopolymers and for determining the mass of molecules and macromolecules, and particularly to large scale biopolymer sequencing and mass determinations in parallel. More specifically, the present invention relates to sequencing of nucleic acids and proteins using an apparatus comprising one or more piezoelectric force sensing elements in an electric or magnetic field.

2. Description of the Background

The principle biopolymers of a cell are polynucleotides such as the nucleic acids DNA and RNA, polypeptides such as proteins and some hormones, and polysaccharides such as starches and mucus. These three polymers constitute over 80% of a cell's dry weight and are involved in all major cellular processes and reactions Many of these biopolymers comprise thousands to millions of atoms linked together in precisely defined spatial arrangements, each arrangement carrying specific information incorporated in its structure. This information constitutes a series of biological messages used to instruct the cell on how to conduct metabolism and how to interact with other cells and molecules. Determining the molecular structure of these biopolymers is an important step in understanding most microbiological processes and numerous methods for determining the structure of both proteins and nucleic acids have been developed.

Conventional techniques for accurately determining polymer structure typically involves some form of electrophoresis. In electrophoresis, migration relies upon the length dependence of drift for specific polymers in a semi-solid. Examples of electrophoresis techniques include agarose gel electrophoresis, polyacrylamide gel electrophoresis and pulsed field gel electrophoresis. When a biopolymer is subjected to an electric field in a hydrogel matrix, such as agarose or acrylamide, its mobility is proportional to

the ratio of net charge to frictional coefficient. This relationship permits the use electrophoresis to obtain information about relative charge for molecules of the same size and shape or about relative size for molecules of the same charge. The most common use of electrophoresis is the separation or qualitative analysis of mixtures based on the sizes and shapes of individual components. Electrophoresis is a powerful and practical, but unfortunately, limited technique. The resolution, speed and accuracy of electrophoresis degrade as the size of the biopolymer increases until the technique become totally useless at about 500 million Daltons. Beyond this size, the drift rate for different length biopolymers is weakly varying and diffusion causes a band broadening in hydrogel supports. In spite of even long electrophoresis times, results are still inadequate.

Conventional solid supports for electrophoresis makes a quantitative analysis of mobility of the biopolymer in a semi-solid. Some supports such as thin-layer cellulose and cellulose acetate interact non-specifically with macromolecular solutes which comprise the semi-solid. Others, such as polyacrylamide, agarose and ion-exchange paper retard the motions of certain biopolymers relative to one another. In conventional electrophoresis, the natural size of the gel pores are orders of magnitude smaller than the size of biopolymer being analyzed. As a consequence, in all such supports, the biopolymer to be analyzed is forced to trace a tortuous path through the support medium which obscures the relationship between the observed net mobility and the actual molecular mobility. The size mobility relationship is further complicated by interactions of the biopolymer with the support. A solid support for electrophoretic analysis of biopolymers, comprising migration paths of known dimensions, is still unavailable. In spite of these limitations, the molecular analysis of biopolymers has advanced considerably. Some of the most significant advances have occurred in sequencing nucleic acids and proteins.

Conventi nal nucleic acid sequencing can be performed either enzymatically and chemically. Although very different in principle, these two

methods are similar in that both generate populations of oligonucleotides that begin from a fixed point and terminate at a predetermined base. Resulting oligonucleotides have lengths which represent at most every residue of the original nucleic acid. Once populations of oligonucleotides are resolved by length, the order of nucleotides along the nucleic acid can be determined. Other methods for nucleic acid sequencing include mass spectrometry, sequencing by hybridization (SBH) and atomic force microscopy. In mass spectrometry, sequence lengths are read by determining the charge-mass ratio of an ionized portion of the nucleic acid. Initial equipment cost is very high and the process requires the talents of numerous skilled personnel. In SBH, a target sequence is hybridized to a plurality of probes with known or determinable sequences and the nucleic acid sequence determined from the pattern of hybridization. In sequencing by atomic force microscopy (AFM), an image of nucleic acid is formed by a sharp probe as it scans over a surface coated with nucleic acids. The sequence of the nucleic acid is determined based on the length of the oligonucleotide and on size of the base's side chain. Only one nucleotide may be analyzed at a time and labor costs tend to be high.

Conventional protein sequencing, although distinct from nucleic acid sequencing, is based on some of the same principles. For example, Edman degradation is a chemical method whereby a protein is repeatedly subjected to a series of degradative chemical reactions. In the Edman process, a protein is exposed to phenylisothiocyanate which covalently binds to a free amino group at an amino terminus of the polypeptide. The complex is then exposed to an anhydrous acid which splits off the N-terminal residue as a phenylthiocarbamoyl-amino acid, leaving the rest of the chain intact. The phenylthiocarbamoyl-amino acid at each step is cycled to the corresponding phenylthiohydantoin derivative and analyzed, usually by gas-liquid chromatography, to determine its identity. The remaining peptide, which is shorter by one amino acid, is subjected to repeated reactions until every amino acid of the peptide has been determined.

Current technology is limited to the analysis of about 20 residues per day. Consequently, a fairly small protein of about 100 residues may require two weeks of skilled labor and expensive equipment to prepare and sequence. This process is still too expensive and too slow for routine or large scale sequencing.

Each of the methods currently available for sequencing of biopolymers have properties that make them less than optimal for sequence determination. The operational cost of currently available methods are high because due to the requirements for skilled labor, expensive equipment, radioactive, fluorescent or hazardous chemicals, and extensive waste management procedures. Moreover, most of these methods sequence in a one-by-one fashion, not practical for large scale sequencing.

Summary of the Invention

The present invention overcomes the problems and disadvantages associated with current strategies and designs and provides novel apparatus and methods for the molecular analysis of biopolymer structure by piezoelectric force sensing.

One embodiment of the invention is directed to a piezoelectric force sensing apparatus (PFSA). The apparatus comprises a plurality of cantilevers to which are attached targets such as biopolymers or biopolymer fragments. Cantilevers are provided with means, such as piezoelectric elements or capacitive sensors, to monitor the amount of stress on the cantilevers which may be induced by mechanical or electromagnetic means. Mechanical stress may be compression, deflection, extension or torque. Electromagnetic stress may be the application of voltage, current, magnetism, electric fields or electromagnetic waves. The amount of stress, manifested as a deflection or distortion on the cantilever, is directly measurable by the piezoelectric elements or capacitive sensors. The PFSA may also comprise positive or negative reference elements. Positive reference elements comprise piezoelectric elements attached to a moiety of known size, charge or mass. Negative or control reference elements comprise

piezoelectric elements without attachments. The use of reference elements provides an internal detector of field strength as well as an internal control in piezoelectric force sensing.

Another embodiment of the invention is directed to methods for analyzing targets such as polypeptides, polysaccharides and polynucleotides using piezoelectric force sensing. Targets or target fragments to be analyzed are attached to a force sensing apparatus. Attached targets are subjected to an electric or magnetic field which generates a resultant force detectable by the apparatus. Force information determined from the multiple lengths of fragments is collected and the structure of the target determined.

Another embodiment of the invention is directed to the detection, isolation and quantitation of the mass of a target. A target to be analyzed is attached to an oscillating piezoelectric element and caused to oscillate electrically. The mass of the target is determined by measuring the frequency response and the resonance frequency of the piezoelectric element. From mass determinations, the target can be isolated and accurately quantitated.

Another embodiment of the invention is directed to methods for sequencing targets such as nucleic acids with piezoelectric force sensing arrays. Nucleic acid sequencing is typically used in medicine and medical diagnosis, in commerce, in defense and in forensic applications. Nucleic acids are isolated from a sample and fragmented, replicated or directly analyzed. The sequence is determined and can be compared to known nucleic acid sequences or to standard sequences for accurate diagnosis of diseases or other disorders, for identification purposes in defense or commerce, or in forensic applications.

Another embodiment of the invention is directed to arrays and kits used in piezoelectric force sensing sequencing. Arrays comprise single- or double-stranded nucleic acids, peptides or other polymers utilized to capture biopolymers or otherwise facilitate piezoelectric force sensing sequencing. Kits

comprise arrays of probes, enzymes, buffers and additional reagents and solutions for determining sequence information by piezoelectric force sensing.

Another embodiment of the invention is directed to methods for measuring target mass using resonance frequency. A small mass or target alters the resonance frequency of a solid, such as a piezoelectric biomorph, to which it is connected at all modes of vibration. As the ratio between the first and second harmonic is fixed, measurement of the first mode of vibration, or harmonic, serves as a reference for the second. Target mass is measured by comparing resonance frequencies before and after attachment, or by analyzing the harmonics of an attached target. This method is independent of temperature, calibration and fabrication quality or measuring technique.

Other embodiments and advantages of the invention are set forth, in part, in the description which follows and, in part, will be obvious from this description and may be learned from practice of the invention.

Description of the Drawings

Figure 1 A force sensing nucleic acid sequencer.

Figure 2 A cantilever for use in a force sensing nucleic acid sequencer.

Description of the Invention

As embodied and broadly described herein, the present invention is directed to methods and apparatus for determining the molecular structure of targets using piezoelectric force sensing and to arrays and kits which can be used in such methods and apparatus.

Structure determination such as sequencing, on both a large and small scale, is critical to many aspects of medicine, agriculture and biology in general such as, for example, in the identification, analysis or diagnosis of diseases and disorders, and in determining relationships between living organisms. Conventional sequencing relies on a unit-by-unit identification f a

biopolymer sequence using electrophoresis in a semi-solid such as a hydrogel (*e.g.* agarose, polyacrylamide or starch). Although attempts have been made to automate polyacrylamide gel electrophoresis analysis, considerable manual manipulation is still required for preparing the gel, loading the biopolymer and analyzing the sequencing information.

Sequence analysis of biopolymers by piezoelectric force sensing provides a fundamentally distinct means to determine sequence information. Sequences are identified collectively in groups and can be analyzed in parallel and continuously. Chips can be specifically designed to function and to operate in parallel arrays. This allows for the simultaneous sequencing of multiple regions of a biopolymer or a mixture of biopolymers from a single sample. These techniques are faster and more efficient than conventional techniques and, as such, more accurate. Multiple analyses can be performed quickly and the results repeated and confirmed.

Rapid sequencing has many applications in basic research and medicine, and in national defense. Organisms with useful characteristics such as cyclic hydrocarbon metabolism may be completely genetically characterized. In medicine, sequencing of the genome of a patient can identify disease genes prior to the appearance of symptoms. Furthermore, an outbreak of a pathogen such as, for example, Ebola virus, can be quickly identified and confirmed and the infected patient or population contained. In national defense, the comparison of sequences from a suspected biological warfare agent with a database of such agents allows for the rapid deployment of effective treatment and measures to prevent harmful effects. Piezoelectric force sensing is broadly applicable to many targets and does not require specific chemicals, enzymes or labels to perform. The process is much faster than conventional procedures, highly accurate and easily automated and computerized.

One embodiment of the invention is directed to a method for determining the molecular structure of a target such as its size, charge, mass,

composition, or sequence by piezoelectric force sensing. Targets or target fragments are attached to piezoelectric elements in a die element. The piezoelectric element may be almost any shape. To facilitate multiple parallel measurements, a plurality of piezoelectric elements may be attached to one substrate to form a die. Substrates may comprise PMOS (p-channel metal oxide semiconductor) or NMOS (n-channel metal oxide semiconductors) materials. The ideal substrate is a nonconductive material which does not interfere with electromagnetic force fields. In a die, plurality of piezoelectric elements may be attached to the substrates in one, two or three dimensions. The attachment of only a small portion of the piezoelectric element to the substrate may increase the sensitivity of the force sensing apparatus.

A target to be tested is attached to another part of the piezoelectric element. The attached target can be labeled such that an applied electric field or magnetic field produces a force on the biopolymer. Suitable labels include, for example, an electric charge or a paramagnetic, a diamagnetic or ferromagnetic moiety. When an electric or a magnetic force field is applied to the biopolymer, a force, proportional to the charge or magnetism of the label is generated. The applied force field stresses and causes a deflection in the piezoelectric element. As the piezoelectric element is deflected, a restoring force is generated proportional to the deflection. Deflection continues under the force field until equilibrium, that is until the restoring force is equal to the applied force. At equilibrium, the stress causes a electric charge differential to accumulate on opposite sides of the piezoelectric element. The charge differential is proportional to stress, which in turn is proportional to the charge or the magnetic moment on the biopolymer. Voltages can be measured by attaching conductors to opposite sides of the piezoelectric element.

Piezoelectricity is electricity or electric polarity, which results from the application of mechanical pressure n a polar dielectric, or electrically non-conductive, or semiconductive substance. Substances which exhibits

piezoelectric effects include ceramics, crystals, polymers, plastics and semiconductors. Some piezoelectric substances are members of more than one group. For example, a piezoelectric crystal may also be a semiconductor. The application of mechanical stress to piezoelectric substances produces an electric polarization (or electric dipole moment per unit volume) proportional to the stress. If the piezoelectric element is isolated, this polarization manifests itself as a voltage across the element and if the surfaces are connected by a conductor, a flow of charge can be observed along the conductor during stress. Conversely, application of a voltage between certain faces of a piezoelectric element produces a mechanical distortion of the element. This reciprocal relationship between voltage and stress is referred to as the piezoelectric effect. The phenomena of generation of a voltage under mechanical stress is referred to as the direct piezoelectric effect and the mechanical strain produced in the crystal under electric stress is called the converse piezoelectric effect. Materials which shows a piezoelectric effect include, for example, tourmaline, ammonium dihydrogen phosphate, ethylenediamine tartrate, barium titanate, quartz, potassium or sodium tartrate and zinc oxide.

The piezoelectric effect can be explained, at least in part, for relatively simple piezoelectric elements such as zincblende (ZnS). In zincblende, every zinc ion is positively charged and located in the center of a regular tetrahedron of four sulphur ions of opposite charge. When zincblende is subjected to a shear stress, some edges of the tetrahedron are elongated while other edges are shortened. As the edges of the tetrahedron become different in length the zinc ion is displaced and this movement gives rise to a electric dipole moment. The dipole moments arising from different repetitive units of the zincblende add up because all have the same orientation. Therefore, a condition for the piezoelectric effect is the absence of a center of symmetry in the crystal structure of an element. Of the 21 crystals classes which lack a center of symmetry, all are piezoelectric with the exception of one.

The PFSA die for measurement in an electric field may also be used for measurements in a magnetic field. A magnetic PFSA die can have a minimal amount or be free of paramagnetic and ferromagnetic materials. Materials which are not substantially influenced by magnetic fields such as copper, lead, quartz, silicone, sodium chloride may be used in construction of the die. A magnetic field may be generated by a permanent magnetic or by an electromagnet. It is fairly straight forward to reach 10 to 20 kilogauss in an iron magnet, and up to 60 to 80 kilogauss in a superconducting magnet. For flexibility in measurements, the magnetic field can be adjusted as desired. Adjustments to the field strength may be performed by changing the distance between the piezoelectric die and the magnet, by changing the current or voltage applied to the electromagnetic, or by applying magnetic shielding between the die and the magnet.

Application of a electric or magnetic field to a piezoelectric element may also generate stress and produce a voltage on the surface of the element. This electric or magnetic field effect can be measured on a PFSA die in the absence of an attached target. The measured field effect can then be taken into account in the calculations for weight, mass and magnetism.

While any piezoelectric element of any shape is suitable for the PFSA, the preferred element is a piezoelectric bimorph. A piezoelectric bimorph comprises at least one and preferably two piezoelectric plates attached in such a way that an applied voltage causes one to expand and the other to contract. The bimorph will bend in proportion to an applied voltage and pressure applied to a bimorph will generate a voltage. The voltage generated is usually proportional to the number of layers of piezoelectric element. Bimorphs can be generated by, for example, micromachining to fabricate a piezoelectric film on top of a silicon cantilever beam. Piezoelectric film may be formed from any piezoelectric chemical such as zinc oxide. The zinc oxide and silicon bimorph may be generated using current semiconductor integrated circuit synthesis technology.

The effectiveness of a piezoelectric element in converting electrical energy into mechanical work, and conversely, converting mechanical work into electrical energy, is a function of its construction. For example, for a zinc oxide-silicon piezoelectric bimorph cantilever, the efficiency is a function of the piezoelectric coupling factor, the thickness ratio and the elastic compliance ratio between the non-piezoelectric and piezoelectric material comprising the bimorph. The sensitivity of the piezoelectric bimorph in the system for measurement is increased if the biopolymer is attached to the free end of the piezoelectric cantilever.

The die can be made of any material which allows an appropriate amount of electromagnetic isolation of individual elements. Suitable substances for the construction of the die include non-conductors and semiconductors and, as such, may be integrated circuits including chips and wafers comprising pluralities of cantilevers. A plurality of cantilevers may be attached to the die for individual measurements. While the cantilevers may be of any shape, a simple shape is preferred for their predictable response to stress during measurement. Examples of simple shape with predictable behavior include elongated cantilevers with a constant cross section, or a plane with constant thickness. The measurement elements, such as the piezoelectric cantilevers, may all be of uniform size and shape to simplify multiple measurements. Alternatively, cantilevers may be utilized in a range of sizes and shapes to measure targets with diverse charge, magnetism or mass.

Cantilevers effective for force detection may be fabricated in a variety of shapes, positions and arrangements. For example, cantilevers may be of an elongated shape, such as straight (planer) or tilted (angled), with a constant or variable cross section. Cantilevers may also be of a planar shape of constant or variable thickness. If the cantilever is a bimorph, the thickness of one or both of the layers may be constant or variable. The cantilevers may also comprise a protective coating to inhibit oxidation, rust, corrosion and chemical reactions

with the targets or the solutions comprising the target. Other complex shapes may be used for the cantilevers such as curves, spirals, helixes and angles. The piezoelectric element may form the cantilever or, alternatively, the piezoelectric element may comprise only a part of the cantilever. A cantilever may also comprise one or more discrete or interconnected elements. These piezoelectric elements may be electrically and mechanically isolated from one another or they may be linked electrically or mechanically. The piezoelectric elements may also form layers, strands, particles or lattices as part of the cantilever and each element may be separately connected to each other element or to the measuring device.

In a PFSA die, a plurality of electrically and magnetically isolated piezoelectric elements may be adjacently attached and each element may be connected through conductors and programmable multiplexers to a digital voltmeter. After the electric or magnetic field is applied, each individual piezoelectric element is addressed through a multiplexer and the voltage on each element individually measured. As such, measurements can be determined in a microscale.

Dies for the PFSA may further comprise magnetic, electric or mechanical stabilizers, or combinations of such stabilizers. Magnetic and electric stabilizers may be passive or active. Passive stabilizers included shielding and ground planes, capacitors, inductors and resistors. Active stabilizers include diodes, transistors, transient suppressors and electromagnetic interference filters. Mechanical stabilizers include active or passive suspension and clamping units which may comprise springs, shock absorbers, friction and oil damping units. Chemical stabilizers include coatings to prevent oxidation, moisture absorptions, reduction or sublimation, or to reduce static electricity.

Polymer-die complexes are placed in a measurement chamber which does not have an initial internal electric or magnetic field. The chamber may be electromagnetically shielded from external fields to reduce background

noise and thereby increase the accuracy of measurements. Static electricity and charged particles are removed from the chamber by proper grounding of all equipment. Initial measurements such as separation distance and response to applied voltage are determined on each piezoelectric member through the multiplexer to determine that each member is performing to specification. Measurements can be repeated at timely intervals to insure that the apparatus has warmed up and is stable. The direction and the strength of the field may be adjusted to determine the optimal measurement parameters. Devices to monitor experimental parameters such as solid state thermometers and hydrometers may also be incorporated into the PFSA chip as a primary or a secondary measurement method to monitor experimental methods.

An electric or magnetic field can be applied either to the whole die or to individual piezoelectric cantilevers. The mass of the attached target or target fragment can be deduced from the voltage generated by the deflected cantilever, the amount of deflection as sensed by the capacitive sensing plates and the voltage needed to restore the piezoelectric cantilever to zero deflection. During measurement, the temperature of the die and the chamber can be adjusted to be within operating limits of the apparatus. Data generated can be stored, for example, in a computer work station, as hard copies or in electronic form. Work stations can be optimized for high throughput sequencing.

Each target will have a characteristic force or mass which can be determined. The mass of each target or fragment can be computed, for example, by any one of three measurements and the separate and independent measurements used to confirm the other methods. The PFSA may also be calibrated by the measurement of targets of known mass. The formula for the mass, deflection and voltage relationship is listed in equation 1. The computation may be performed on site or, alternatively, to decrease cost or allow for portability, measurement and data collection equipment may be assembled in

one site and the data stored and forwarded by conventional means to a central site for analysis.

Reference measurement elements positioned near the point of actual measurement may be utilized to keep interference from appearing as a normal reading. For example, an unexpected change in the electric or magnetic field during measurement may affect the reference and the measurement elements equally. If the voltage measurement is directed to the difference between the reference and measurement elements, the change in electric or magnetic field may not significantly affect the output. The pair of signals, from the reference and the measurement electrodes is measured and the voltage difference recorded. An amplifier may be used to amplify the electric signal before measurement. Suitable amplifiers may be a differential amplifier having a high input impedance, good rejection of common-mode interference and preferably low-noise-voltage and low-drift with a stable adjustable output offset added. Because the input is multiplexed and a number of cantilevers are measured in quick succession, the amplifier may also have good high frequency performance in the range of kilohertz to megahertz to gigahertz. Any degradation of high frequency response may be negated as much as possible by active compensation through positive feedback. The response of the measurement device may be enhanced by placing the amplifier and measurement device as close as possible to the PFSA die.

The multiplexer and the amplifier may be micromachined as an integral part of the PFSA die. Additional electronic devices which may be integrated into the PFSA die include an analog to digital converter and operational amplifiers. To maintain a stable operating temperature, the measurement chamber may be temperature controlled and, in addition, the PFSA die may be bonded to coils which carry a heating or cooling fluid or gas. The PFSA die may also be optionally bonded to objects with a large thermal capacity to reduce the rate of temperature fluctuation.

The electric field may be measured by precise voltage measurement of the conductors applying the field. Magnetic fields may be measured by a Hall effect magnetometer, a flip coil magnetometer, a nuclear magnetic resonance magnetometer, a flux gate magnetometer or a superconducting quantum interference device (SQUID).

For piezoelectric force sensing to be practically applied to target analysis, movement of the cantilevers needs to be sensitively and accurately measured. One sensitive method for measurement of deflections and displacements is to use a capacitive transducer. To produce a capacitive transducer on the PFSA die, two adjacent regions, one on the piezoelectric element and one on the substrate are coated with a conductor to form a capacitor. The conductor may be electrically isolated from the piezoelectric element with an insulator. The formed capacitor is connected to a multiplexer so it can be connected to external measuring devices. The conductor, insulator and connectors may be micromachined onto the PFSA die. By making the capacitor part of a resonant circuit or by using a high frequency alternating current bridge, very small changes in position may be measured. A number of methods may be implemented to determine the deflection (Horowitz and Hill, *The Art of Electronics*, Hamilton Printing Company, Rensselaer, NY, 1989). For example, the capacitor may be charged through a resistor with a first bias voltage. The change in capacitance due to deflection can be amplified using a low impedance output operational amplifier made from low noise field effect transistors. To improve sensitivity and linearity, the low impedance output may be biased before it is read with a sensitive analog to digital converter or voltmeter. The digital output of the voltage is then sent to the central processing unit for analysis.

Targets which can be analyzed include polymers such as plastics, petroleum products, synthetic polymers and the like, and natural, recombinant or synthetic molecules or macromolecules including biopolymers such as nucleic acids (*e.g.* DNA, RNA), proteins, lipids, polysaccharides and biopolymer

homologs such as PNA. Targets may also be particles such as molecules, macromolecules, supramolecules, viruses, cells and whole tissues. Depending on the size of the target and the capabilities of the sensing apparatus, the first step in structure determination using piezoelectric force sensing can be fragmenting the target into sizes which can be easily analyzed. Fragments of polymers are typically between about 5 to about 1,000 monomers in length, preferably between about 10 to about 500 monomers, and more preferably between about 25 to about 100 monomers in length. However, a very wide variety of lengths may be quickly and accurately analyzed. For example, one of ordinary skill in the art could easily analyze fragments of nucleic acids less than about 5 bases, and greater than about 1,000 bases, and fragments of proteins less than about 5 amino acids and greater than about 100 amino acids in length. Fragments can be generated enzymatically, mechanically or chemically. Most methods produce nonspecific fragments over a range of sizes depending only on the harshness of the fragmentation process. Alternatively, specific fragment sizes can be created if, for example, convenient enzymes (*e.g.* nucleases, proteases, hydrolases, peptidases) and other cleavage sites are available. Modifications to the biopolymers may be removed by glycosidase and phosphotases. Alternatively, arrays of detectors may be used to detect patterns of force and those patterns analyzed to determine the structure of the target. This is particularly useful with regard to particles such as cells.

Targets may be attached to the die intact or fragmented into multiple fragments which are subsequently attached to the die. For example, fragments of DNA can be created using polymerase chain reaction (PCR) elongation or partial endonuclease or exonuclease digestion. Fragments may also be created by replicating a target nucleic acid in the presence of modified or unmodified nucleotides, or nucleotides comprising boron or a mixture of each type of nucleotide. Digestion of the boronated target with exonuclease III produces a nested set f fragments. Fragments of proteins can be created by

chemical degradation or partial or complete protease digestion. Any degradation process can be used including chemicals such as hydrochloric acid and sodium hydroxide, radiation or physical cleavage. Enzymes which can be used for enzymatic digestion include, for example, trypsin, chymotrypsin and any protease. As the process does not require that the ends produced be identical or even that the fragments be similar, any process known to fragment a polymer can be utilized.

Targets which are fragmented into identical or at least similar lengths may be directly analyzed. Biopolymers comprise subunits of different mass and, as a result, the length, mass and sequence of the biopolymer are interrelated. The sequence of the biopolymer may be determined from its mass and/or length. Analysis of a collection of biopolymers such as nucleic acids or polypeptides by piezoelectric force sensing can be used to determine sequence information. Depending on the method, the need to specifically capture and analyze single target biopolymers is reduced or eliminated. Pluralities of dies containing pluralities of cantilevers can be used to capture and determine the sequence of biopolymer fragments in parallel and continuously as sample containing target flows past cantilevers.

Targets can be attached to dies via covalent or non-covalent bonds such as electrostatic or hydrogen bonds. For example, attachment may involve coupling agents such as streptavidin-biotin, avidin-biotin, cell adhesion molecules, antigen-antibody interactions, aptamer-substance interactions, nucleic acid hybridization, covalent chemical coupling or any method known to those of ordinary skill in the art to attach a polymer to a solid support. The attachment may also include a spacer molecule to facilitate the sensing process, for example, by providing an internal reference standard, or to facilitate subsequent removal of targets from the die.

Targets may be obtained from recombinant or other man-made or synthetic sources, or purified from natural sources such as plants, animals

including humans, environmental or microbial sources, or from pathological samples. Preferably, target biopolymers are polypeptides, polynucleotides or polysaccharides and may be linear, branched or particularized. Polynucleotides, such as DNA, RNA, polynucleotide amides (*e.g.* polymers of 2-aminoethylglycine), and modified polyamides (*e.g.* polythioamides, polysulphinamide, polysulphonamide), comprise the bases purines, pyrimidines and purine and pyrimidine derivatives and modifications, which are linearly linked to a chemical backbone. Modifications of nucleic acid may include methylation and phosphorylation. Common chemical backbone structures are deoxyribose phosphate, ribose phosphate, and polyamide. The purines of both DNA and RNA are adenine (A) and guanine (G). Others that are known to exist include inosine, xanthine, hypoxanthine, 2,6-diaminopurine, and other modified bases. The pyrimidines are cytosine (C), which is common to both DNA and RNA, uracil (U) found predominantly in RNA, and thymidine (T) which occurs almost exclusively in DNA. Some of the more atypical pyrimidines include methylcytosine, hydroxymethyl-cytosine, methyluracil, hydroxymethyluracil, dihydroxypentyluracil, and other base modifications. These bases interact in a complementary fashion to form base-pairs, such as, for example, guanine with cytosine and adenine with thymidine. There may also be non-traditional base pairing such as Hoogsteen base pairing which has been identified in certain tRNA molecules and triple helix structures.

Polypeptides comprise amino acid residues including the α-amino acids which have an amino group (NH_2) and a carboxylic group (CO_2H) attached to the same carbon. There are a great many naturally occurring amino acids and amino acid derivatives which differ in side chain structure. In fact, there are over 300 naturally or synthetically modified amino acids (Fasman, *CRC Practical Handbook of Biochemistry and Molecular Biology*, CRC Press, Cleveland OH, 1990). Amino acids can be grouped into the aliphatic amino acids including glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), and isoleucine (Ile), the

aromatic amino acids including phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), the aliphatic hydroxyl amino acids including serine (Ser), and threonine (Thr), the basic amino acids including lysine (Lys), arginine (Arg), and histidine (His), the acidic amino acids including aspartate (Asp) and glutamate (Glu), and the amide side chain amino acids including asparagine (Asn) and glutamine (Gln), the sulfur containing amino acids including cysteine (Cys) and Methionine (Met) and proline (Pro) and alanine (Ala) which has a secondary amino group. These amino acids may also be modified by natural or artificial means. Typical modifications of amino acids include glycosylation, methylation, and phosphorylation.

Alternate types of target biopolymers that may be sequenced using piezoelectric force sensing include lipids, fatty acids and man-made biopolymers such as peptide nucleic acids and peptides of nonprotein origin. Nonprotein peptides usually differ structurally from those derived from proteins. For example, the tripeptide glutathione, found in all cells of higher animals contains a glutamic acid residue joined in an unusual peptide linkage involving its γ-carboxyl rather than the α-carboxyl group. Other examples of nonprotein peptides include the muscle dipeptide carnosine, the antibiotic tyrocidin A, gramicidin, and valinomycin, and the hormones oxytocin, vasopressin, bradykinin and thyrotropic releasing factor.

Targets may be labeled with detectable labels such as, for example, a radioactive or luminescent moiety or a charged or magnetic or paramagnetic moiety. Alternatively, labels may be an inherent property of the target, such as the charge of a nucleotide, or a detectable moiety attached to the target. Targets may also be labeled by bombardment with electromagnetic waves to cause a charge, or an ion to develop on the target. Labeled moieties are detected simultaneously or sequentially with th piezoelectric force sensing. For example, using cysteines as labels for protein sequencing, all the cysteines may be labeled with a radioactive moiety through their sulfhydryl groups. After

sequence determination by PFSA, the biopolymers with cysteines can be detected by contacting the PFSA die to a radiation detector. This additional sequence information may be used to confirm or to help determine protein sequence.

Another embodiment of the invention is directed to a piezoelectric force sensing apparatus. Such an apparatus is depicted in Figure 1 as biopolymer sequencer **100**. This apparatus comprises a target sequencer work station **110** and at least one PFSA die **120**. Target sequencer work station **110** and at least one PFSA die **120** are connected via lines **150**.

Target sequencer work station **110** comprises a computer **102**, a modem **104**, and a PFSA interface **106**. Target sequencer work station **110** may also comprise a printer, oscilloscope, plotter etc. for displaying charts and graphs, for example. Computer **102** may be any type of computer such as mainframe, work stations, microprocessors, personal computer, dedicated computer hardware, or a supercomputer. Computer **102** may comprise a multiple computers working in parallel or a single machine comprising parallel processors. In addition, dedicated hardware processors designed for PFSA calculation may also be used. Computer **102** may also comprise means for data storage such as, for example, magnetic, optical, or semiconductor memory.

PFSA die **120** comprises control electronics unit **108**, control lines **112**, and cantilever **114** all provided on a PMOS substrate **122** (Figure 2). Control electronics unit **108** comprises drivers **116** and a multiplexer **118**. Multiplexer **118** may have a range of between about four to one to about 1024 to 1 or greater and may be uni-directional or bi-directional. Control lines **112** connect cantilever **114** to control electronics unit **108**. Cantilever **114** may comprises an array of cantilevers, each comprising its own piezoelectric force sensing element or plurality of elements that are controlled by controlled by one or more electronics units **108**. In one embodiment, the array may comprise an array of 16 by 16 elements, or 256, individually addressable elements. For more

sensitive uses, a larger number of elements may be used, such as 128 x 128 or 256 x 256 or 1024 x 1024. Many other array sizes may also be used.

The PFSA die may be reused after a measurement. For example, the attached target may be removed chemically or enzymatically depending on the nature of the attachment. Disulfide attachments are removed under reducing conditions, and most protein bonds can be released under denaturing conditions. The die can be washed with an acid or basic solution to further remove non-specific binding. If the targets were removed with minimal disruption of the attachment moiety, the die can be used immediately. If the removal method destroyed the site of target attachment, a new attachment moiety has to be reattached before the die can be reused. The washing of the PFSA die does not have to be complete. Before reuse the die may be placed in the measurement chamber and each cantilever may be tested and any residual charge or magnetism can be recorded. This background charge or magnetism can be subtracted from subsequent measurements. Alternatively, the die may be constructed of an inexpensive material such as silicon and safely disposed of after one or only a few uses.

Attachment of the target and the measurement of charge, magnetism or mass may also be performed at different times. The target may be attached to the PFSA die in the field or in a clinic and mailed under suitable protection to a central processing center for measurement. After measurement and processing, the central processing center may clean and reuse the die.

Cantilever **114** is depicted in greater detail in Figure 2. PMOS substrate **122** serves as the base for the cantilever structure. On top of PMOS substrate **122** is a grounded capacitive sensing plate **124**. Space **126** separates grounded capacitive sensing plate **124** from a beam capacitive sensing plate **128**. Space **126** is provided by a support structure **130**. On top of beam capacitive sensing plate **128** is a PZT bimorph **132**.

Cantilever **114** may be made as follows: PMOS substrate **122** is fabricated. Piezoelectric layers (capacitive sensing plates **124** and **128** and bimorph **132**) are applied to PMOS substrate **122**. Piezoelectric layers can be deposited by micromachining.

PFSA target sequencer **100** may operate as follows: First, at least one target sequence **140** is attached to cantilever **114**. Because each PFSA die **120** may have a plurality of cantilevers **114**, multiple target sequences may be attached to PFSA die **120**, each target sequence being attached to one cantilever **114**. Cantilever **114** and target sequence **140** are placed in the presence of an electric or magnetic field **142**. In the presence of electric or magnetic field **142**, target sequence **140** generates a force on cantilever **114**. A measurable opposite force **144** is then applied by control electronics unit **108** via control lines **110**. Opposite force **144** may be sensed and input to biopolymer sequencer work station **102**. As described later, opposite force **144** indicates the length of target sequence **140**. The data from the PFSA may be stored in a data storage medium in work station **102** first and analyzed at a later time. Alternatively, work station **102** may transmit the data to another computer for analysis. Off site data analysis may reduce the cost and the bulk of a PFSA system and allow the construction of portable PFSA systems.

The electric field may be generated by placing the piezoelectric element with the attached target between two conductors carrying opposite charges. The conductors may be induced to carry opposite charges by attachment to a power supply with an adjustable voltage. The voltage of the power supply, and hence the charge on the conductor may be controlled by computer work station **102**. Conductors may also be mounted on adjustable mounts so that the distance may be varied either by manual or by computer control. The shape of the conductors may be varied depending on the type of EMF field desired. For example, the conductors may be two parallel plates for the generation of a uniform electric field, two opposing pins for the generation

of a field which varies with distance from the center, two parallel wires or a wire and a parallel plate to generate a field which varies with one axis, but is constant in another axis. The separation distance of the two conductors may be variable, so that the movement of the piezoelectric element causes a change in the EMF to the element. EMF applied to the piezoelectric element may be adjusted by changing the voltage or altering the separation of the two conductors, either manually or automatically by computer.

To control the generation of corona, arcing and electric wind, the field strength supplied by the electrical conductors can be monitored and controlled. To prevent the charging of particles by the electric field for non-aqueous samples or aqueous samples that are stable when dried, measurements may be taken in vacuum or, otherwise, in substantially particulate-free air. A vacuum may be achieved by performing the measurements in a chamber evacuated of air with one or more vacuum pumps. A combination of low vacuum pumps and high vacuum pumps attached to the reaction chamber may be used to establish and maintain a pressure of, for example, 10^{-7} torr or less. A completely or substantially particulate-free air environment may be achieved by filtering the air around the measurement site or by flushing the measurement chamber with particulate-free air.

Typically, electrical and mechanical instruments are allowed to reach a stable operating temperature. A stable operating temperature may range from near absolute zero (-273°C) for superconducting magnets to room temperature or to over 100°C. Preferably, operation temperatures are at or near room temperature although different parts of the PFSA may require different operating temperatures. Periodic measurements of control samples can be made to insure there is no drifting of the readings or breach of the electromagnetic shielding. Cooling or heating of the measurement area may be applied to maintain all equipment within operating limits. Instrumentation may be shielded from external electromagnetic influence such as capacitive coupling, magnetic

coupling and radio frequency coupling to increase consistency and accuracy of measurements. Also, electrical cabling can be shielded electrically as well as magnetically. Movement of conductors under electric or magnetic fields generates a current within the conductor. Therefore, after the electric field is applied, movement of the PFSA die can be kept to a minimum. Any induced current can be compensated for during the data analysis. Alternatively, measurements may be performed under a uniform electric or magnetic field which requires no movement of the die. Measurements can also be made after movement has ceased and the induced current has subsided.

Length of a target, such as a biopolymer, is directly related to the amount of force each biopolymer sequence applies in response to an electric or magnetic field. Therefore, in a preferred embodiment, the length of the biopolymer is determined by sensing the force that the sequence applies in response to an electric or magnetic field. In general, multiple biopolymers are analyzed in parallel to increase throughput and speed of the analysis and because the multiple measurement may be used to statistically improve the accuracy of the method.

The apparatus of Figure 1 may be used in a preferred embodiment to determine the force and, thus, length and identity of the various sequences in the biopolymer fragments. A protein which shows high specificity and affinity to another molecule such as streptavidin to biotin, monoclonal and polyclonal antibodies to antigen, or cell adhesion molecules may be covalently attached to a microspot on the surface of cantilever 114. Streptavidin and biotin are particularly suitable. Preferably, streptavidin is attached to each element of the piezoelectric array.

After streptavidin has been attached to cantilever **114**, cantilever **114** may be contacted to the nested biopolymer fragments. In this manner, the different fragments attach to the streptavidin on cantilever **114**. One array may be used for the biopolymers of one reaction or the biopolymers from multiple

related or unrelated sequences may be placed on one chip. Additionally, multiple cantilevers **114** may be used for each reaction to ensure that each of the lengths in the mixture attaches to at least one element on one cantilever. Instead of using streptavidin, the sequences may be directly attached to cantilever **114**. The cantilever may be derivatized chemically for the covalent attachment of biomolecules.

As depicted in Figure 1, once the lengths are attached to cantilever **114**, cantilever **114** is subjected to an electric or magnetic field. The sequence **140** generates a force resulting in a deflection on cantilever **114** equal to the product of the charge of one electron per, for example, nucleotide base, the number of bases, and the electric or magnetic field. This deflection, γ, is depicted in Figure 1. Circuitry in the PMOS substrate applies an equal opposing voltage to the piezoelectric cantilever **114** and removes the deflection. The amount of initial deflection and the removal of deflection may be monitored on the capacitive sensing plates **124** and **128**. The electric or magnetic field and the restoring voltage may be applied and measured at steady state or transiently. For the measurement, both the electric or magnetic field and the applied voltage may be varied.

The deflection is related to the force generated by the sequences by the electric field and the voltage applied to the piezoelectric cantilever substrate. The following equation represents this relationship:

$$\gamma = AF + BV \quad (equation\ 1)$$

When the deflection is removed, $\gamma = 0$, and then this equation reduces to:

$$V = -\left(\frac{A}{B}\right) F \quad (equation\ 2)$$

Because the force, F, is equal to the product of the electric field (E), the charge of an electron (1 e-), and the number of polymer monomers such as nucleotides of DNA in the sequence (n), this equation becomes:

$$V = -\left(\frac{A}{B}\right) E\, n\, (e-) \quad (\textit{equation } 3)$$

The electric field, E, and the charge of an electron, e- are known. Therefore, by measuring the voltage applied, the number of nucleotides in the sequence is then known. Measuring the voltage applied may be performed using equations expressed in the following matrix:

$$\begin{bmatrix} \alpha \\ \delta \\ v \\ Q \end{bmatrix} = \begin{bmatrix} \frac{3S_{11}L}{2wh^3} & \frac{3S_{11}L^2}{4wh^3} & \frac{S_{11}L^3}{2h^3} & \frac{3d_{31}L}{2h^2} \\ \frac{3S_{11}L^2}{4wh^3} & \frac{S_{11}L^3}{2wh^3} & \frac{3S_{11}L^4}{16h^3} & \frac{3d_{31}L^2}{4h^2} \\ \frac{S_{11}L^3}{4h^3} & \frac{3S_{11}L^4}{16h^3} & \frac{3wS_{11}L^5}{40h^3} & \frac{d_{31}wL^3}{4h^2} \\ \frac{3d_{31}L}{2h^2} & \frac{3d_{31}L^2}{4h^2} & \frac{d_{31}wL^3}{4h^2} & \frac{2\epsilon_{33}Lw}{h}\left(1-\frac{k_{31}^2}{4}\right) \end{bmatrix} \begin{bmatrix} M \\ F \\ P \\ V \end{bmatrix} \quad (\textit{equation } 4)$$

α = slope at end of cantilever **114**
δ = deflection at end
v = displaced volume
Q = charge
S_{11} = elastic compliance
ϵ_{33} = dielectric constant
t = beam to ground spacing
w = bimorph width

M = moment at end of cantilever **114**
F = force at end
P = uniform load
V = applied voltage
d_{31} = piezoelectric coefficient
k_{31} = coupling coefficient
h = bimorph height
L = bimorph length

Voltage measurements using equation 4 may be analyzed with biopolymer sequencer work station **110** and particularly computer **102**. Target

sequencer work station collects all of the data generated by cantilevers **114** and, thus, amasses the data into the lengths present in each cantilever array. The target sequence may be identified with the knowledge of the lengths of the targets using algorithms commonly used in the analysis of traditional sequencing data. For example, the data from a PFSA analysis can be analyzed using the same method as the analysis of a DNA sequencing gel. Target sequencing using PFSA may begin by looking for the shortest length and building the sequence identity with longer and longer lengths.

Target sequencing can be performed in either direction along a particular target. The original sequence may be determined by performing the inverse operation, the complement operation or both. The specific operation depends on the attachment site (*e.g.* 5'-; 3'-; N-terminal; C-terminal) and the method of ladder generation (*e.g.* polymerase elongation or chemical degradation). A common limitation shared by all methods of nucleic acid sequencing is the inability to determine the identity of the last base. This can be overcome by sequencing the complementary strand, by sequencing overlapping segments or by ligating a known nucleic acid to one end. These techniques are well-known to those of ordinary skill in the art and many are described in *Current Protocols in Molecular Biology* (Eds., F.M. Ausubel et al., Wiley Interscience, 1989).

Another embodiment of the invention is directed to a method for diagnosing a disease or a pathogen by the presence or absence of a characteristic target. The target can be isolated from a sample suspected to contain a pathogen or the isolated pathogen may be analyzed directly. Targets may be proteins, nucleic acids, fatty acids, oils or any material specific to the disorder. Samples may be obtained from animals such as bodily fluids or tissues, plants such as biomass or environmental sources such as bodies of water or soil. Samples may also be obtained from food sources to detect possible contamination by harmful parasite, bacteria or virus. If for example, the detection method is by nucleic

acid sequencing, the sample may be treated to remove proteins and lipids by protease treatment followed by phenol and/or chloroform extraction. The remaining nucleic acid may be sequenced using a primer specific for pathogen sequence. Alternatively, the isolated sample may be amplified by polymerase chain reaction. The resultant DNA is attached to the PFSA chip and analyzed. Sequence information or sequence length is determined and compared to a database with sequence information from known pathogens for identification. This method is particularly useful for the detection of pathogens with multiple substrains including bacteria such as mycoplasma and viruses such as the human immunodeficiency viruses (HIV), the human papillomaviruses (HPV) and the hepatitis viruses. A knowledge of the particular substrain is useful to determine the course of treatment as well as for epidemiological and public health studies.

Molecular analysis of targets has a great many practical utilities. For example, targets may be analyzed for forensic purposes such as in the identification of evidence, in the placement of individuals at crime scenes or in connecting or reconstructing crimes. Targets including nucleic acids and proteins can be isolated, sequenced and compared to other targets from other samples or to reference libraries to identify or determine genetic relationships. This method can also be used for prenatal diagnosis such as amniotic fluid and chorionic villus sampling. Piezoelectric force sensing can also be used to sequence patient genomes for the detection of genetic defects. Diseases that may be detected include thalassemia and sickle cell anemia, hemophilia, Lesch-Nyhan syndrome, phenylketonuria, familial hypercholesterolemia, Huntington's disease, certain mental disorders, pseudohypoparathyroidism and some forms of cancers. These and other detectable disorders may have a single or small number of unique mutations or groups of mutations such as abnormal numbers of repeated sequences. The list of disorders which can be diagnosed by biopolymer sequencing increases with the increase in understanding of the genetic basis of many diseases increases.

Another embodiment of the invention is directed to the profiling of target by an analysis of their subunits. Targets such as polypeptides, polynucleotides, and polysaccharides, for example, may be hydrolyzed to their component monomer subunits. This mixture of subunits may be attached to a PFSA die and the relative percentages of each subunit may be profiled and biopolymer fingerprints obtained. Because biopolymers may be very heterogeneous, a subunit profile can be very informative. For example, ribonuclease lacks tryptophan, fibrous proteins such as fibroin and collagen lack several amino acids, over 90% of the amino acid residues of elastin are nonpolar, lysozyme, histones and cytochrome c have predominately basic groups, and pepsin have predominately acidic groups.

Other examples of specific amino acids which are characteristic of different organisms may be found in standard biochemistry manuals (*e.g. CRC Practical Handbook of Biochemistry and Molecular Biology*, Fasman ed., CRC Press, Boca Raton, FL, 1990). Nucleic acids of pathogens such as viruses and bacteria have GC content between 0.7 to 0.3 while the GC content in humans in generally less than 0.4. Suitable compounds for hydrolysis of protein include hydrochloric acid and sodium hydroxide while suitable enzymes for hydrolysis of nucleic acid include DNase and RNase. After hydrolysis, the reaction is neutralized and contacted to the piezoelectric force sensing element to attach individual amino acids, nucleotide or saccaharides to each cantilever. The profile of the individual components can then be determined under an electric or magnetic field with the conjugated piezoelectric element.

Another embodiment of the invention is directed to a method to rapidly read a DNA sequence which has been added to another material. DNA molecules are chemically and environmentally stable and can be routinely detected at the single molecule level by th use of sensitive amplification techniques such as PCR. While PCR is rapid, it can only detect the length and not the sequence of a DNA label. Utilizing piezoelectric force sensing, specific

DNA sequences can be used as an additive to provide a unique identifier for almost any material. For example, a 40 basepair DNA label can encode 40 messages based on length or 10^{40} messages based on a sequence. Piezoelectric force sensing DNA sequencing can overcome the current sequencing cost and speed limitations and make DNA labeling by sequence an economically viable technology. DNA labeling and piezoelectric force sensing detection may be implemented by using an oligonucleotide synthesizer to produce a DNA label. This label could be added to a substance such as crude oil. To determine the origin of oil from a spill or from the hold of a ship, the nucleic acid can be extracted by chain amplification techniques and sequenced. The sequence can be compared with a database of known DNA labels to determine the origins of the oil sample.

Materials which can be identified may be solids, liquids, gasses, semi-solids and combinations thereof. Such materials include bodies of water, soils, purified or semi-purified chemicals such as oil or other petroleum products, paints, powders, paper or other wood products and any manufactured or purified material. Specific DNA additives may also be used to trace the diffusion or origin of sensitive or controlled materials. Such small quantities would be needed that it would be virtually undetectable on the material being identified. Further, as nucleic acids are fairly ubiquitous, an individual would be unable to determine if the specific sequence detected was the identifier or just nucleic acid sequences of the normal flora microorganisms. One would have to know where to look and exactly what sequence to look for. Specific DNA sequences could be added to paper, to military hardware, preferably in the form of lubricating grease or oil, or simply on the hardware itself, to computer parts such as chips, to imported or exported goods or even to foods. Both natural and synthetic DNA is generally safe and n n-toxic and can even be consumed. Thus, the sequence and even the presence and location of the identified sequence on the object can be confidential.

Biopolymers which can be sequenced include biopolymers important in commerce or defense. For defense purposes, a PFSA die may be used to sequence samples from biological facilities to ensure compliance with treaties relating to biotechnology. Samples of air, soil and water may be collected and analyzed during routine inspection. The sequencing of the complete genome or proteins of these organisms may allow their genetic manipulation to enhance their desirable genetic qualities.

Another embodiment of the invention is directed to the measurement of targets in a PFSA die under both an electric and a magnetic field. PFSA may be used in assays in which one target is labeled with a charge while a second target is labeled with magnetism. Both targets may be attached to the PFSA die together and the measurement for the charge or the magnetism may be made individually or simultaneously. This method allows the use of the PFSA die for two measurements before it is recycled and hence can double the throughput of the system. Furthermore, assays for two different targets may be made at once. For example, a DNA sequence may be labeled with a charge and attached to the PFSA die. A DNA binding protein may be labeled magnetically and applied to the attached DNA. Where the protein attached, the PFSA die senses both a charge and a magnetism. DNA which do not bind protein are charged, but not magnetized. An array of DNA with different sequences may be attached to a PFSA die and the attachment of a magnetically labeled protein quickly determines the sequence specificity of the protein.

The magnetic field and the electric field may be applied to the attached target in two orthogonal directions. The distortion of the cantilever measured indicates the amount of electrical charge and magnetism of the target simultaneously. The two force fields need not be completely orthogonal, any interference of the two force fi lds on each other may be subtracted mathematically.

Another embodiment of the invention is directed to measurement of force without the use of piezoelectricity. Cantilevers may be micromachined into a die using materials which would be elastic under measurement condition. Most materials, made sufficiently small and thin may become elastic under measurement conditions. Targets labeled by charge or magnetism may be attached to the non-piezoelectric cantilevers using the same method of attachment as with the PFSA dies. The non-piezoelectric cantilever is equipped with capacitive sensing plates to measure the deflections generated by the applied magnetic or electric field. The relationship between deflection, charge, magnetism or applied force can be calculated by the known physical parameter of the materials involved. Alternatively, a series of targets of known charge, magnetism, and size may be attached and measured to determine the response of the die. As with the PFSA dies, a series of reference cantilevers without target attached can be used as internal standards to reduce the effects of noise and to monitor the consistency of the applied fields.

Another embodiment of the invention is directed to methods for measuring target mass by analysis of resonance frequency. A target attached to a material alters the resonance frequency of that material, such as a piezoelectric biomorph, at all modes of vibration. As the ratio between the first and second mode of vibration or harmonic is fixed, measurement of the first harmonic serves as a reference for the second. Target mass is measured by exciting an element attached to a target to create a frequency response, determining a resonant frequency from the frequency response, and calculating target mass from the resonant frequency by comparing resonance frequencies with a corresponding unattached element or by analyzing the frequency response itself. This method is independent of temperature, calibration and fabrication quality or measuring technique.

A frequency response is a measure of the effectiveness with which an element, transmits the different frequencies of periodic force applied to it.

The excitation force applied is not limited to mechanical force but may be any force to which the element responds. For example, if the element has piezoelectric properties, the applied force may be an electric current or voltage. If the element is paramagnetic, diamagnetic or ferromagnetic, the applied forced may be a magnetic field. Furthermore, the element may be driven by pressure waves such as sound. A combination of driving forces may be used to measure the frequency response.

The excitation force, once applied, will result in oscillations in the element. A number of physical parameters may oscillate in response to the force including position, displacement, swing, pressure, strain, current, voltage, resistance, admittance, impedance, and capacitance. When the excitation force reaches the natural resonance frequency of the element or its harmonics, the response may become large relative to the response at non-resonant frequencies. Depending on the physical attribute monitored, a large response may be reflected in a sudden increase or decrease of a physical variable. For example, displacement, swings, or admittance may increase and impedance may decrease.

Resonance frequencies are inherent properties of the physical, as known to those of ordinary skill, are unique characteristic of every element. Elements may possess multiple resonance frequencies, namely the fundamental resonance frequency and its harmonics. One physical characteristic that affects the resonance frequency is the mass of the element. Thus, the attachment of a target to an element will alter both the mass, the fundamental resonance frequency and its harmonics. By measuring and comparing the frequency of an element with and without an attached target, the amount of change in frequency may be recorded. The magnitude of the change in resonance frequency is mathematically related to the mass and thus may be directly calculated. Furthermore the ratio of frequencies of any two harmonic frequencies of an element also varies with the attached targets is also mathematical related to the mass of the target. Thus, the mass may also be deduced from any two harm nic

resonance frequencies of the element. The two harmonic frequencies may be any two such as first and third, first and second, second and third, but some are easier to measure because of their speed.

All elements have a degree of elasticity and possess resonance frequencies. Preferably, the element is a solid because solids are compressable. As such, solids may act as oscillators when harmonically driven to resonate. The more useful oscillating solids possess low damping coefficients for ease of oscillation. Oscillating solids may be comprised of a variety of materials including ceramics, glass, polymers (*e.g.* plastics, resins, starches, gels such as acrylamide or agarose), metals (*e.g.* alloys, electroplated materials, LIGA processed materials), insulators, crystals, semiconductors and combinations of these materials. Semiconductors are crystallin materials whose electrical conductivity is intermediate between that of a metal and that of an insulator. A large number of substances are known as semiconductors including germanium, silicon, gray (crystalline) tin, selenium, tellurium and boron. Germanium and silicon, two of the most used semiconductors, belong to group IV of the periodic table of the elements and have crystal structures similar to diamond. Semiconductor substances of type AB comprise elements from columns symmetrically placed with respect to group IV. Other semiconductor substances include indium antimonide (InSb), cadmium telluride (CdTe) and silver iodide (AgI), which are examples of groups III-V, II-IV and I-VI semiconducting elements.

Materials that exhibit piezoelectric properties may be driven to oscillate electrically. One of the variables that influence the resonance frequency is the mass of the oscillator. As elements are attached to targets at their surface, the mass of the element is increased by the mass of the attached target. A PFSA die, for example, may have a plurality of piezoelectric el ments each of which is individually addressable with a multiplexer for connection to matched oscillator circuits with a reference piezoelectric element. The reference

piezoelectric element can be identical to the measurement piezoelectric element except for the absence of an attached target. Both the reference and the measurement element are induced to oscillate at resonant frequency electronically. The resonant frequency of each of the piezoelectric elements are electronically compared to derive the mass of the attached target.

Solids may be excited to vibrate and to resonate by the application of an oscillating force. In a solid comprising one or more piezoelectric elements, the driving force may be an oscillating voltage applied to the one or more piezoelectric elements. The oscillating voltage is preferably a sinusoidal wave but other form of cyclic wave functions such as sawtooth waves, step waves, may also be used. The driving wave may be generated by analog circuits, by digitally controlled oscillators or by a programmable digital-to-analog converter. Resonance frequency may be the fundamental frequency of the solid or from higher order harmonics (second, third, fourth and higher harmonics) of the fundamental frequency.

A cantilever comprising one or more piezoelectric elements always has some degree of elasticity and, consequently, a natural spectrum of vibrational modes. Cantilevers with different elasticities in two axes, may resonate at a different frequency depending on whether the transverse displacement is along the more rigid or the more flexible axis. A cantilever with a flat shape and elongated cross section may be expected to have this behavior. The characteristic vibrations of a piezoelectric cantilever subjected to an oscillating driving force may be driven independently in multiple modes including compression, bending and shearing. Each mode of vibration may exist independently of each other and have its own fundamental resonant frequency and harmonics. The driving force may be changed to selectively activate one mode of vibration. If desired, each mode may be driven independently or together. Furthermore, th choice of elements may affect the amount of coupling between the modes.

For example, to drive the cantilever into a second order harmonic vibration, a cantilever may comprise two discrete subunits connected to the electrical driving force in opposite polarity. When voltage is applied, one part of the cantilever will be displaced in one direction while another part will be displaced in the opposite direction. Where the oscillating piezoelectric element is a bimorph made from two piezoelectric strips joined over their entire length, the resonance frequency of a bimorph is precisely determined by the equation:

$$1+\cos \Omega L \cosh \Omega L = 0 \quad (\textit{equation 5})$$

In this system, the first resonance occurs at $\Omega L = 1.87504$, the second at $\Omega L = 4.6941$, the third at $\Omega L = 7.8547$. A typical property of these resonance frequencies is that they can be measured with great accuracy.

When a mass is attached to the bimorph, it alters the resonance frequency of all the modes of vibration in a predictable manner. From this change in frequency, the mass can be calculated from equations 5 and 6 using basic trigonometry and calculus. In a measurement, an alternating current applied to the element over a range of frequencies range and the admittance of the piezoelectric material over this frequency range is recorded. At the fundamental resonance frequency (also referred to as the first harmonic), a peak of increased admittance (1/impedance) or decreased impedance (1/admittance), may be detected. Other peaks may be detected as the alternating current approaches higher harmonics frequencies. In this manner the first, second, third, fourth, fifth and higher harmonics of a piezoelectric material may be determined and recorded. The frequency of the alternating current at these peaks of admittance will correspond directly to the resonance frequencies of the piezoelectric element.

While a mass may be attached at any location on the bimorph, in some applications the alteration of resonance is more effective if the mass is

attached to the end of a piezoelectric element. Optimal placement of the attached mass depends on the harmonic frequency and the desired sensitivity and range. In a first harmonic vibration, the mass may be placed at the end for greater accuracy or in the middle or near the supported end for increased range. In a second harmonic vibration, the mass may be placed closer to a node for maximum range or closer to an antinode for maximum sensitivity. Thus, one or more masses may be attached or placed at any longitudinal and latitudinal position(s) along the cantilever or at either end of the cantilever vibrating in the second harmonic. By the same reasoning, one or more masses may be attached or placed at any longitudinal or latitudinal position(s) along the cantilever or at either end of the cantilever vibrating in the third harmonic. This analysis can be continued to a fourth, fifth and higher harmonic frequency, as desired.

The addition of mass changes the resonance frequency, but does not affect the accuracy of resonance frequency determination. The relationship between the altered resonance frequency and the mass may be deduced from analytical calculations or experimentation. This relationship allows the determination of the resonant frequency from which the mass may be derived with great accuracy. As shown by equation 5, the first and second harmonic both change due to the mass, but change in a different fashion, which makes their ratio a unique identifier for the mass. The resonant frequency taking into account of higher order harmonics is determined by the equation:

$$1 + \cos \Omega L \cosh \Omega L + \mu(\cos \Omega L \sinh \Omega L - \sin \Omega L \cosh \Omega L) = 0$$

(equation 6)

in which μ is the reduced mass, $m/\rho A$, where m is the mass to be measure and ρA is the mass of an infinitesimally thin cross section of the bimorph. Piezoelectric elements may be driven to their first and higher order harmonics by altering their driving frequency or by altering the number of oscillators. Because this is a linear system, a bimorph made by using piezoelectric thin films on

silicon substrates can be combined with two on board oscillators which may drive the bimorphs in both resonance modes simultaneously. Similarly, these bimorphs may be driven to the third, fourth or higher level harmonics by increasing the driving frequency or by attaching more on board oscillators.

This analysis is particularly amenable to piezoelectric applications. For example, piezoelectric elements to be used for specific harmonic applications can be inexpensively produced and easily manufactured in mass quantities. PFSAs comprising resonating elements may be used for military, environmental, industrial and biomedical applications. As such, PFSAs comprising resonating elements may also be used to measure the presence of target elements such as gases, toxic chemicals, pollutants, industrial by products, mass of cells. In this capacity, a chemical which reacts with a target element may be attached to the end of a piezoelectric bimorph. When the bimorph is expose to the particular target element, the chemical will react. The mass and ultimately the resonance frequency of the piezoelectric element is altered. Target elements may be any measurable chemical such as CO_2, CO, H_2, NO containing compounds, ammonia, metal fumes, lead, combustion gasses from diesel or gasoline engines, pollutants, industrial products and by-products, and chemical warfare agents. Any chemical which reacts with the target element may be attached to the end of the piezoelectric element. Any reaction which alters the mass of the attached chemical may be used including, for example, absorption, hydrolysis, oxidation and reduction. Such methods and devices could be used to measure indoor or outdoor air quality, combustion efficiency and the presence or absence of certain chemicals.

PFSAs can also be easily utilized to measure the mass of an attached target. Target mass is determined by a comparison of the resonance frequencies of a cantilever before and after attachment to a target. Alternatively, the resonant frequencies of two cantilevers, ne attached to a target and one

unattached, may be measured in parallel to determine target mass. In a PFSA die, multiple measurements of mass may be performed in parallel.

The mass of a target can also be determined by measuring the resonant frequencies of multiple harmonics of a piezoelectric oscillator. For example, target is attached to a piezoelectric element and the first and the second harmonic resonance frequencies measured. As the ratio between the first and the second harmonic is fixed by the mathematical expression relating these altered frequencies, the measurement of the first harmonic serves as the reference for the second harmonic. Other harmonics (*e.g.* third, fourth, etc.) may also be compared to determine mass. The ratio of the first and second harmonic frequency become the variable that determines the mass. One advantage of this method is that changes in temperature will affect both the first and the second harmonic such that the ratio will remain the same. This method is temperature independent and no calibration of the bimorph is necessary. The actual accuracy with which the bimorph is fabricated is immaterial as only the ratio between difference harmonic is of importance. The independence of this method with regards to temperature, calibration and fabrication quality is a significant advantage in its manufacture, in use and in maintenance. Also, the method is gravity independent and, thus, PFSA devices will operate in environments of changing acceleration and g-force. These environment include moving vehicles such as cars, ships, planes, low and zero gravity environments such as space vehicles, and sensitive electronic instruments.

The following experiments are offered to illustrate embodiments of the invention and should not be viewed as limiting the scope of the invention.

Examples

Example 1 Generation of a Nested Set of Biotinylated Oligonucleotides.

A nested set f biotinylated oligonucleotides is generated by polymerase chain reaction elongation. A test oligonucleotide containing a target

sequence to be analyzed is used as a template. A primer oligonucleotide is also required in addition to the target sequence because DNA polymerases typically cannot initiate new chain growth, but require a primer with a 3' OH group. A second oligonucleotide, biotinylated at the 5' terminus and containing a sequence complementary to 6 nucleotide at the 3' terminus of the test oligonucleotide, is used as a sequencing primer.

The primer oligonucleotide is dissolved at a concentration of 0.3 pmole/μl in 10 mM Tris-HCl, pH 7.4, 5 mM NaCl, 0.1 mM EDTA, pH 8.0. Target oligo nucleotides are dissolved at a concentration of 0.5 mg/ml in 10 mM Tris-HCl, pH 7.4, and 0.1 mM EDTA. Forty μl of the primer solution is mixed with 8 μl of DNA solution to anneal primer to target. Annealing is accomplished by incubating the mixture at 55°C for 30 minutes and allowing the mixture to cool to 15°C in 30 minutes. The mixture is divided into 4 tubes, each with a respective chain terminating nucleotide (ddTTP, ddCTP, ddGTP or ddATP). Dideoxy chain termination reactions are initiated by the addition of 2.5 μl of the appropriate ddNTP solution, 7.5 μl of the dNTP solution and 6 units of DNA polymerase Klenow fragment to each of the four tubes. After 12 minutes of incubation at 37°C, the reaction is terminated with the addition of 5 μl of 250 mM EDTA, pH 8.

Example 2 Attachment of Biopolymers to the Force Sensing Array.

Fragment products of the individual dideoxy chain termination reactions from Example 1 are contacted directly to a PFSA die and each PFSA die is connected with a computer. Individual elements of the array are individually addressed by the computer through a multiplexer. The beam to ground spacing of each cantilever of the array is tested individually. A voltage is applied to the piezoelectric element and the beam to ground spacing for each cantilever is tested again. The location of any elements which failed the test by

not responding to the applied voltage are noted and the output from these failed element are not considered in future calculations.

A single streptavidin molecule attached to each cantilever. The individual dideoxy chain elongation products are contacted to the PFSA die to allow the biotin to attach to the streptavidin. The die with the attached nested DNA ladder is washed with 10 mM Tris-HCl, pH 7.2, 1 mM EDTA.

Example 3 Sequencing of Attached Biopolymers.

Each PFSA die with the attached biopolymer is placed in a chamber without an electric field. Beam to ground spacing is measured for each cantilever through the capacitive sensing plates as individual elements of the PFSA are addressed individually through the multiplexer. A voltage is applied to each cantilever until the deflection of the piezoelectric cantilever is eliminated. This position is reached when the capacitive sensing plates shows the same reading as the initial measurement in the absence of the electric field. The voltage required to restore the position of each cantilever are recorded and stored in the computer.

Example 4 Data Analysis.

Data generated from the sequencing reactions of Example 3 is collected in work station 102. Biopolymer lengths are calculated according to equation 4. The sequence of the target is identified because the biopolymer sequencer work station 102 also has input indicating from which of the mixtures the length determinations were derived. By looking for the shortest length, work station 102 identifies the length from the ddATP mixture, thus indicating that the first base in the complement sequence. This procedure is followed until the sequence is determined. The complete sequence is determined and can be inverted, complemented and added to the primer sequence to derive the target

sequence. The last base of the biopolymer can be determined, if necessary, by sequencing opposing strands and overlapping regions.

Example 5 Sequencing of a Polypeptide.

A target polypeptide is digested separately with trypsin and chymotrypsin and the resulting digestion products separated by thin layer chromatography. Peptide fragments are resuspended in 50 mM NH_4HCOOH, pH 7.85, in the absence of calcium and magnesium. A column comprising immobilized trypsin and a column comprising immobilized chymotrypsin are prepared for use by washing with 20 column volume of 50 mM NH_4HCOOH, pH 7. The target sequence is applied over each column and incubated for 1 hour at 37°C. Digested peptides are recovered from the immobilized enzyme by draining the column solution.

Peptide fragments are separated and isolated with a C18 high performance liquid chromatography column (Millipore; Bedford, MA) using a gradient of aqueous trifluoroacetic acid in methanol (Aldrich Chem.; Milwaukee, WI). Fragments are placed in 6 N hydrochloric acid at 100°C for 10 hours to reduce peptide fragments to amino acids. The resulting amino acid solution is neutralized with 1 M HEPES buffer until the pH is 7.5. Following hydrolysis, amino acids are conjugated to biotin to facilitate conjugation to the PFSA. Biotinylation is started by the addition of a 10 molar excess of NHS-Biotin dissolved in dimethyl sulfoxide to the hydrolyzed peptide for 10 minutes at room temperature. Biotinylated amino acids are layered onto a streptavidin PFSA. The measurement provides an amino acid profile.

The ratio of the oligopeptide indicates the length of the oligopeptide or multiples of the oligopeptide. Trypsin cleaves at the carboxyl side of lysine and arginine and, thus, the trypsin digest shows the amino acids contiguous with Lys at the carboxyl terminus. Chymotrypsin cleaves

preferentially on the carboxyl side of aromatic and other bulky nonpolar residues. The order of peptide is determined from an analysis of the tryptic fragments.

Other embodiments and uses of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. The specification and examples should be considered exemplary only with the true scope and spirit of the invention indicated by the following claims.

We Claim:

1. A method for determining structure of a target comprising the steps of:

a) attaching the target or fragments of said target to a piezoelectric element;

b) applying a force field to said target;

c) detecting a deflection of said piezoelectric element;

d) applying a voltage to the piezoelectric element to neutralize said deflection; and

e) determining the structure of said target.

2. The method of claim 1 wherein the target is a biopolymer.

3. The method of claim 2 wherein the biopolymer is a polynucleotide, polypeptide or polysaccharide.

4. The method of claim 3 wherein the polynucleotide is DNA, RNA or PNA.

5. The method of claim 1 wherein the target or target fragments are modified.

6. The method of claim 5 wherein the target or target fragments are modified by phosphorylation, methylation, glycosylation or a combination thereof.

7. The method of claim 1 wherein the target or target fragments are polymers between about 10 to about 100 monomers in length.

8. The method of claim 1 further comprising the step of fragmenting the target wherein each fragment comprises a sequence that corresponds to a sequence of said target.

9. The method of claim 8 wherein the target fragments comprise a nested set.

10. The method of claim 8 wherein the target fragments are generated by physical or enzymatic cleavag of said target.

11. The method of claim 10 wherein enzymatic cleavage comprises subjecting said target to an enzyme selected from the group consisting of nucleases, polymerases, glycosidase and peptidases.

12. The method of claim 8 wherein the target fragments are between about 10 to about 100 monomers in length.

13. The method of claim 1 wherein the target or target fragments are attached to the piezoelectric element via coupling agents.

14. The method of claim 13 wherein the coupling agents are selected from the group consisting of avidin, streptavidin, biotin, cell adhesion molecules, antibodies, aptamers, nucleic acids, antigens and combinations thereof.

15. The method of claim 1 wherein the target or target fragments are attached to biotin and the piezoelectric element is attached to streptavidin.

16. The method of claim 1 wherein the force field is magnetic, electric or a combination thereof.

17. The combination force field of claim 16 wherein the magnetic and the electric fields are applied at different directions.

18. The method of claim 1 wherein the strength of the force field is adjustable.

19. The method of claim 1 wherein the direction of the force field is adjustable.

20. The method of claim 1 wherein the piezoelectric element is selected from the group consisting of ceramics, crystals, plastics and combination thereof.

21. The method of claim 1 wherein the piezoelectric element comprises a semiconductor.

22. The method of claim 1 wherein the piezoelectric element is a bimorph.

23. The method of claim 22 wherein the bimorph comprises silicon and zinc oxide.

24. The method of claim 1 wherein the structure determined is the sequence, size, composition, charge or mass of the target.

25. A method for sequencing a target comprising the steps of:

a) attaching the target or fragments of said target to a piezoelectric element;

b) applying a force field to the target or target fragments to produce a deflection of said piezoelectric element;

c) detecting a voltage from said piezoelectric element caused by the deflection; and

d) determining the sequence of said target.

26. The method of claim 25 further comprising the step of fragmenting the target and attaching the fragments to multiple piezoelectric elements.

27. The method of claim 25 wherein the target fragments are about the same length.

28. A method for determining charge or magnetism of a target comprising the steps of:

a) attaching the target or fragments of said targets to a piezoelectric element;

b) applying a force field to the piezoelectric element;

c) detecting a deflection of the piezoelectric element;

d) applying a voltage to the deflected piezoelectric element to neutralize said deflection; and

e) determining the charge or the magnetism of the target.

29. A method for determining a mass of a target biopolymer comprising the steps of:

a) attaching the target biopolymer or fragments of said target biopolymer to a piezoelectric element;

b) applying a force to said piezoelectric element;

c) detecting a deflection of said piezoelectric element; and

d) determining the mass of said target biopolymer.

30. The method of claim 29 wherein the target biopolymer is selected from the group consisting of proteins, nucleic acid, polysaccharides and lipids.

31. The method of claim 29 wherein the target biopolymer is attached to the piezoelectric element by covalent or noncovalent interactions.

32. The method of claim 29 wherein the force applied is mechanical, electrical, magnetic or pressure.

33. The method of claim 29 wherein the deflection is detected by a change in the conductance of the piezoielectric element.

34. A method for determining a mass of a molecular or macromolecular target comprising the steps of:

a) contacting a target to an element;

b) exciting the element to create a frequency response;

c) determining a resonant frequency from the frequency response; and

c) calculating the mass of the target.

35. The method of claim 34 wherein the target is attached to the element by covalent or noncovalent interactions.

36. The method of claim 34 wherein the element is piezoelectric.

37. The method of claim 34 wherein the element is excited by applying an electrical, mechanical, pressure or magnetic force, or a combination thereof, to the element.

38. The method of claim 34 wherein the frequency response is a measure of admittance, capacitance, oscillation amplitude, oscillation frequency, displacement, pressure, strain or combinations thereof, of the element.

39. The method of claim 34 wherein the mass is determined by comparing the ratio of the resonant frequencies at different modes of resonance.

40. A piezoelectric force sensing apparatus (PFSA) die for parallel determination of mass, charge or magnetism of a target comprising:

a solid support;

a plurality of piezoelectric elements attached to said support forming a plurality of cantilevers;

means for individually measuring deflection stress of each of said cantilevers; and

means for applying a voltage to said plurality of cantilevers.

41. The PFSA die of claim 40 wherein the cantilevers are arranged in one, two or three dimensions.

42. The PFSA die of claim 40 wherein the means for measuring deflection is selected from the group consisting of capacitive sensing plates, piezoelectric elements or a combination thereof.

43. The PFSA die of claim 40 wherein the measuring means detect deflection of the piezoelectric element in at least two dimensions.

44. The PFSA die of claim 40 further comprising means to detect the cantilever in the non deflected position.

45. A piezoelectric force sensing element for the determination of mass and charge of a target comprising:

a solid support;

a cantilever comprising a piezoelectric material;

means for measuring stress of said piezoelectric material; and

means for applying a voltage to said piezoelectric material.

46. A piezoelectric force sensing array for the determination of mass and charge of a target comprising:

a solid support;

a plurality of cantilevers comprising a piezoelectric material;

means for measuring the deflection of each said plurality of cantilevers; and

means for applying a voltage to said plurality of cantilever.

47. A piezoelectric force sensing apparatus (PFSA) comprising:

a computer:

a plurality of bidirectional multiplexers each with a plurality of input and output pins wherein each output pin is electrically connected to said computer;

a plurality of cantilevers comprising a piezoelectric element wherein each of said plurality of cantilevers is electrically connected to each input pin of the multiplexer;

means for generating a force field;

means for measuring the deflection of each said plurality of piezoelectric cantilevers; and

means for applying a voltage to said plurality of piezoelectric cantilever.

48. The PFSA of claim 47 wherein the computer is one or more mainframe computers, computer work stations, microprocessors or dedicated computer hardware.

49. The PFSA of claim 47 wherein the force field is a electric field, a magnetic field or a combination thereof.

50. The PFSA of claim 47 wherein the means for measuring stress comprise capacitive sensing plates, piezoelectric elements or a combination thereof.

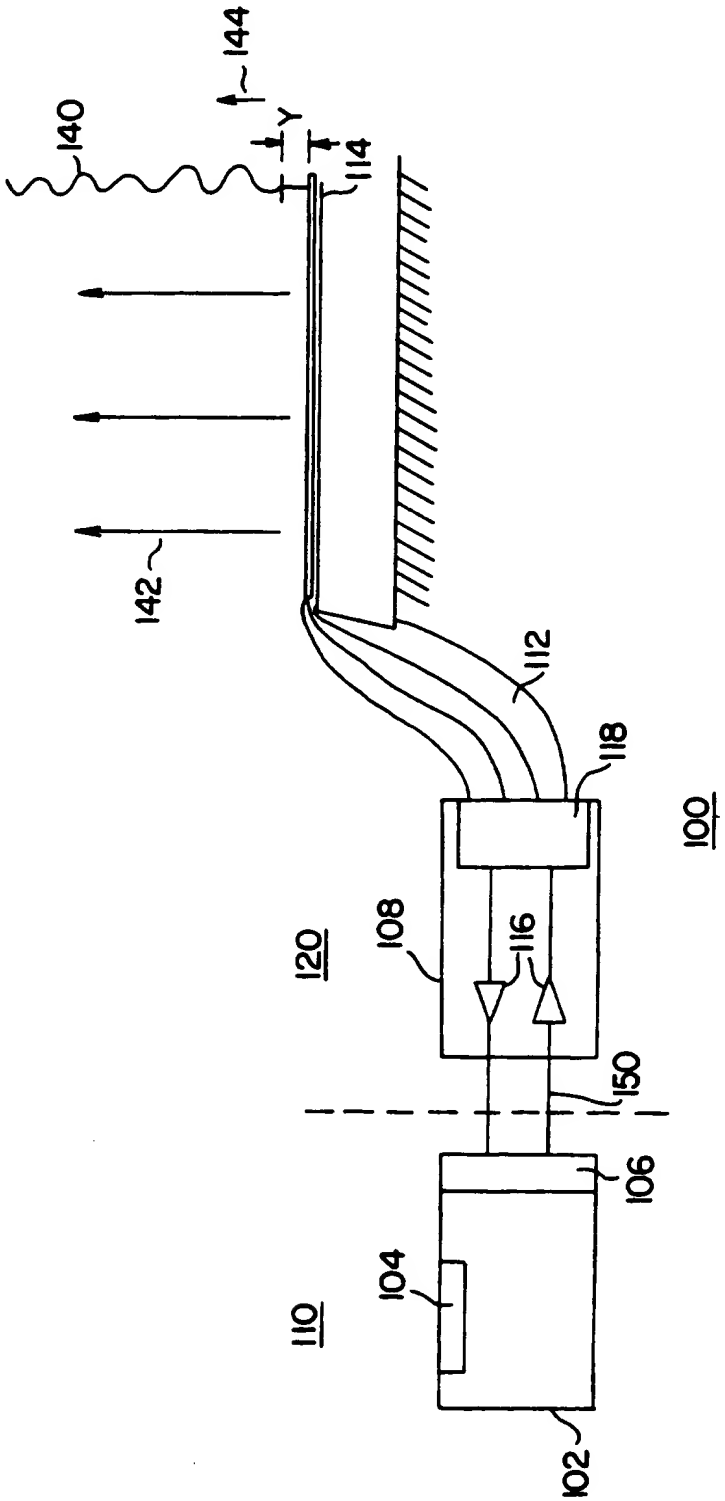

FIG. 1

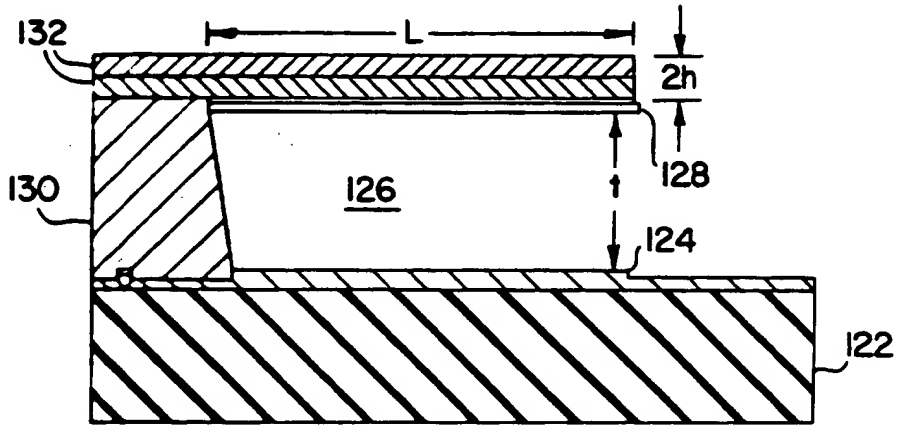

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/14462

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01B7/34 C12Q1/68 G01N33/68 C07K1/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01B C12Q G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	8TH INTERNATIONAL CONFERENCE ON SCANNING TUNNELING MICROSCOPY/ SPECTROSCOPY AND RELATED TECHNIQUES, SNOWMASS, CO, USA, 25-29 JULY 1995, vol. 14, no. 2, ISSN 0734-211X, JOURNAL OF VACUUM SCIENCE & TECHNOLOGY B (MICROELECTRONICS AND NANOMETER STRUCTURES), MARCH-APRIL 1996, AIP FOR AMERICAN VACUUM SOC, USA, pages 789-793, XP002021241 BASELT D R ET AL: "Biosensor based on force microscope technology" see the whole document --- -/--	1-14, 16-21, 24-27, 29-50

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 December 1996	14.01.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Brock, T

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/14462

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	see the whole document	34-38

Y	US,A,4 835 461 (SNELLING CHRISTOPHER) 30 May 1989	28
A	see the whole document	1,25,29, 34,40, 45-47

X	WO,A,91 05261 (DU PONT) 18 April 1991	34-38
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	see the whole document	

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/14462

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